

Short Communications

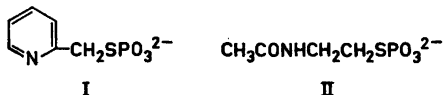
Synthesis of Dilithium S- α -Picolylphosphorothioate and Barium N-Acetylcysteamine S-Phosphate

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It was shown in previous reports^{1,2} that several S-substituted phosphorothioic acids were effective as radioprotective agents when given to mice prior to irradiation. In order to investigate further the effect of substituents in the carbon chain linked to the phosphorothioate group several new substances have been prepared, two of which are described in this communication.

Trilithium phosphorothioate reacts smoothly and quantitatively at pH 9 with a slight excess of α -picolylchloride giving S- α -picolylphosphorothioate (I). The reaction is not catalyzed by dimethyl formamide. This is expected since the effect earlier observed of dimethyl formamide in this type of reactions can be linked to a cyclization of the participating aminoalkylhalogenide.³ Obviously an intermediary step involving a bicyclic aziridinium type compound is not likely in the case of α -picolyl chloride.



Attempts to prepare N-acetylcysteamine S-phosphate (II) by acetylating cysteamine S-phosphate with acetic anhydride or with acetyl chloride in aqueous solution proved unsuccessful due to the lability of the S-P bond under these conditions. It was

found, however, that sodium hydrogen cysteamine S-phosphate is easily soluble in glacial formic acid and in this solvent the amino group of the compound can easily be acetylated by acetic anhydride at room temperature. Both the phosphorothioate and the amino group of the substance could theoretically become acetylated during this operation. However, the fact that the isolated barium salt was found to contain one atom of barium per phosphorothioate group shows that it only consists of N-acetylated material. The homogeneity of the substance was confirmed by paper chromatography.⁴

Experimental. Dilithium S- α -picolyl phosphorothioate dihydrate. 60 mmole of α -picolylchloride hydrochloride and 50 mmole of trilithium phosphorothioate were dissolved in 100 ml of water and pH was adjusted to 9 (glass electrode) with a saturated solution of lithium hydroxide monohydrate in water. After 90 min of stirring the silver ion test for unreacted phosphorothioate ions⁴ gave a negative result. An excess of lithium hydroxide solution was then added, giving a pH of about 11, followed by 300 ml of ethanol. The precipitate was filtered off and washed with ethanol. 10.1 g (80 %) of substance were thus obtained. (Found: C 28.6; H 3.4; S 12.5; P 12.4. Calc. for $\text{C}_6\text{H}_4\text{NCH}_2\text{SPO}_3\text{Li}_2 \cdot 2\text{H}_2\text{O}$ (253.1): C 28.5; H 3.4; S 12.7; P 12.2).

Barium N-acetylcysteamine S-phosphate dihydrate. 25 mmole of sodium hydrogen cysteamine S-phosphate⁵ were dissolved in 25 ml of glacial formic acid and 25 mmole of acetic anhydride were added. The solution was evaporated to dryness in vacuum in a rotating evaporator (bath temperature: 20-25°). The remainder was dissolved in 25 ml of water buffered with about 1 g of tris(hydroxymethyl) aminoethane of pH 8-9. 30 mmole of barium acetate was added under stirring in small portions, the pH being maintained at 8-9 with 10 M sodium hydroxide. A precipitate was centrifuged off and discarded. To the clear supernatant was added 75 ml of methanol. The crystalline

precipitate was filtered off and washed with methanol. Yield: [4.5 g (49 %)]. (Found: C 12.8; H 3.2; S 8.6; P 8.4; Ba 37.9. Calc. for $\text{CH}_3\text{CONHCH}_2\text{CH}_2\text{SPO}_3\text{Ba}\cdot 2\text{H}_2\text{O}$ (370.6): C 13.0; H 3.3; S 8.65; P 8.36; Ba 37.1).

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The Identity of Cyanein and Brefeldin A

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Cyanein is a crystalline antibiotic isolated from *Penicillium cyaneum*¹ and possessing antifungal,^{1,2} anti-HeLa cell,³ and anti-nematodal⁴ activity. It also inhibits the growth of the root tips of *Allium cepa*⁵ and depresses the mitotic activity in the root tips of *Vicia faba*.⁶

Cyanein possesses the composition $\text{C}_{16}\text{H}_{24}\text{O}_4$ (mol.wt. by mass spectrometry: 280) and its oxygen atoms were accounted

for by the presence of a lactone grouping and two secondary alcohol functions, which were readily acetylated to give a crystalline diacetate. On catalytic hydrogenation (Pd/C) cyanein afforded a crystalline tetrahydro-derivative. The NMR-spectrum (in $(\text{CD}_3)_2\text{SO}$) revealed the presence in cyanein of the grouping $\text{CH}_2\text{CH}(\text{O}-)$, the system $-\text{CH}=\text{CH}-\text{CO}-\text{O}$, as well as an isolated double bond.

At this point of the structure work, Sigg⁷ published the structure of brefeldin A, an antibiotic produced by *Penicillium brefeldianum*,⁸ and identical with decumbin,⁹ previously isolated from *Penicillium decumbens* and preliminarily characterized as 'toxic to rats and to goldfish, and inhibitory to wheat germination, but not an active antibiotic under the tested conditions.'¹⁰

On critical comparison of the published data⁷ for brefeldin A, its diacetate, tetrahydro-derivative, and the diacetate of the latter, with those of the corresponding cyanein-derivatives, we can now conclude that cyanein is, in fact, identical with brefeldin A.

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